IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Miri Seiberg et al.	Docket No. JBP0430USCIP1
Serial No.	:	10/659,598	Art Unit: 1618
Filed	:	September 10, 2003	Examiner: Shirley V. Gembeh
For	:	**METHODS FOR TREATING SKIN CONDITIONS**	

DECLARATION OF MIRI SEIBERG, PH.D.

I, Miri Seiberg, am a Distinguished Research Fellow in the Skin Research Center at Johnson & Johnson Consumer Companies, Inc. My education includes a Ph.D. in Molecular Biology from The Weizmann Institute of Science, Rehovot, Israel, in collaboration with Princeton University, Princeton, NJ and a B. S. in Life Sciences from Tel-Aviv University, Tel-Aviv, Israel.

1. Ethylene oxide (ETO) is an agent known to cause protein denaturation. As summarized in the book "Validation of Pharmaceutical Processes: Sterile Products", Second Edition by Carleton (Hardcover - Nov. 5, 1998), p.367, section 3, (a copy of which is attached hereto as Exhibit A) sterilization by ETO is based on the chemical reactions between ETO and proteins, which modify the proteins so "...their modifications by ETO will disrupt or destroy the protein's activity". Thus, one of ordinary skill in the art at the time the invention was made would have known that exposure of soy protein isolate powder to ETO, as set forth in the Jin publication, would have caused the denaturation of proteins existing in the soy protein isolate.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by

fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

MIRI SEIBERG, PH.D.

8/10/10

DATE

Exhibit A

VALIDATION of PHARMACEUTICAL PROCESSES

Sterile Products

Second Edition, Revised and Expanded

edited by

Frederick J. Carleton

Carleton Technologies, Inc.
Boynton Beach, Florida

James P. Agalloco

Agalloco & Associates
Belle Mead, New Jersey

MARCEL DEKKER, INC.

NEW YORK • BASEL

A. METHOD SELECTION

Selection of an appropriate sterilization method is based on such items as the effect of the method on product quality or esthetics, regulatory requirements, industry practice, the economics of the sterilization process, and the logistics of sterilization compared with those of the overall manufacturing process [7–10]. For example, thermolabile liquids are typically sterilized by filtration. The guiding phrase in the regulated manufacture of drugs and medical devices is *safe and effective*. These simple words form the basis of a complex network of statutes and regulations that dictate many of the practices and procedures employed in the pharmaceutical industry. Any sterilization method that compromises the safety or the effectiveness of the product is precluded from use. There are situations, however, when a property of the product unrelated to either safety or effectiveness may be altered by a given sterilization method. To illustrate this, consider plastics, which are commonly used in the production of medical devices. The sterilization methods typically applied to these devices are either ethylene oxide or ionizing radiation. Some plastics, when exposed to high doses of ionizing radiation, will discolor. Although this may not affect the performance of a device, it can result in a cosmetic change that may be unacceptable. In this circumstance, ethylene oxide may then become the method of choice.

Clearly, an important consideration in manufacturing processes is the economic effect of each process step on the cost of the final product. Thus, process development personnel strive to choose steps, or unit operations that are cost-effective. When the option of more than one sterilization method is open to the process engineer, he or she will usually select the least costly method (in terms of price, time, or test requirements). Moreover, any one sterilization method's economics can be optimized (minimized) by proper design of the sterilization process. For example, in sterile filtration, different combinations of prefilters with the final sterilizing filter can change the economics of the process. Generally, prefilters are cheaper than sterilizing filters, so the most economical system uses sufficient prefiltration to prevent plugging of the final filter.

B. MECHANISMS OF DESTRUCTION AND REMOVAL

In the broadest sense, all sterilization methods perform identically; they all render products sterile. Close examination of the individual methods demonstrates that each operates in a different manner. This section describes the biological basis of microbial removal or destruction for each of the five major methods of sterilization: moist heat, dry heat, sterilant gas or vapor, ionizing radiation, and filtration.

1. Moist Heat

Moist heat, or steam under pressure, is the most studied method of sterilization. First developed by Pasteur and Chamberlain, in the late 1800s, it has been characterized in terms of its operational parameters, predictability, and mode of action. As with all sterilization methods, the cellular function that is of primary interest is reproduction (no reproduction means no growth). The reproductive process of microorganisms is directed by nucleic acids (DNA and RNA) and mediated by enzymes—protein biocatalysts—which, among other things, direct nucleic acid synthesis and the construction of cellular components. Generally speaking, the three-dimensional (tertiary) structure of proteins, especially enzymes, determines their function. This structure is the result of the primary linear arrangement of amino acids, each of which has different chemical properties. When proteins are formed, the sequence of amino acids dictates the shape of the protein. This is because the individual amino acids

interact with each other and their aqueous environment to yield the most stable shape. If the shape of the protein is changed after its formation (e.g., through protein denaturation), then its function changes. Often this change is irreversible and results in nonfunctionality. Cooked egg whites are an example of irreversible protein denaturation.

During moist heat sterilization, it is the irreversible denaturation of vital enzymes that results in cell death. Both water vapor and elevated temperature are required to effectively denature proteins (kill cells). If water vapor is present, much lower temperatures are required in heat sterilization. Generally, moist-heat sterilization is performed with water-saturated steam under pressure [15 psig (1.03 bar), 121.1°C]. The water vapor contributes to the available heat at any temperature (e.g., saturated steam at 121.1°C provides at least seven times as much available heat as air at the same temperature). This alone, however, does not explain the efficiency of kill for moist heat. The water vapor also interacts directly with the protein at the elevated temperature to denature it. The exact protein or proteins that are rendered nonfunctional by moist-heat sterilization are largely unknown, but from a practical standpoint, this is unimportant. The process of cell destruction is predictable and reproducible under defined conditions of operation.

2. Dry Heat

Dry heat is used to sterilize materials, such as glassware, metal parts, dry powders, and other substances, that can withstand heat. The lack of moisture in this sterilization process, compared with moist-heat sterilization, necessitates higher temperatures, on the order of 160°–170°C, and longer time periods. Sterilization by dry heat actually represents an incineration process during which the cells are destroyed. This is generally considered an oxidative process during which the cellular components are destroyed at elevated temperatures. Other factors that may play a role include water content, water location within the spore or cell, and possible effects on DNA. Again, the exact site of action is unknown, but the process is predictable and reliable.

3. Sterilant Gas or Vapor

Sterilant gases and vapors, such as ethylene oxide (EtO), formaldehyde, vaporous hydrogen peroxide (VHP), and peracetic acid, are used for a variety of applications in aseptic processing. Gaseous and vaporous sterilants are most often used for heat-labile or radiation-incompatible plastic containers, closures, and drug delivery systems, as well as for sterility test isolators, manufacturing isolator systems, lyophilizers, and medical devices. Because ethylene oxide and VHP are the most commonly encountered gaseous or vaporous sterilants in aseptic processing, their microbiocidal effects are described in this section.

Ethylene oxide is the most commonly used gaseous sterilant. It works through a chemical reaction with cellular components, such as nucleic acids and macromolecules. Its chemical activity is as an alkylating agent, and it is through this mechanism, acting on nucleic acids, that it is thought to kill cells. EtO replaces labile hydrogen atoms with hydroxy ethyl ($-CH_2CH_2OH$) groups. Macromolecules, such as proteins contain functional groups, such as carboxyl ($-COOH$), hydroxyl ($-OH$), sulfhydryl ($-SH$), and amino ($-NH_2$), the hydrogens of which are labile to alkylation. Because many of these groups play an important role in protein structure and function, their modification by EtO will disrupt or destroy the protein's activity. If the protein is vital to cell replication, then death occurs after activity is lost. Interestingly, some studies have shown EtO to be almost as effective against spores as it is against vegetative cells. The predictive results of EtO on spores of *Bacillus subtilis* var. *niger* are the basis of this bacterium being chosen as the indicator microorganism for this method of sterilization.

The vaporous phase of liquid hydrogen peroxide (H_2O_2; VHP) is widely used for the surface sterilization of isolators and, when optimized with pulsed vacuum cycles, can be used to sterilize certain types of packaging components. VHP cannot be used as a sterilant for liquids owing to condensation.

The cidal mechanism of VHP is attributed to the oxidation of sulfhydryl groups (–SH) and double bonds in proteins, lipids, and surface membranes. Hydroxyl radicals are produced by the following reaction:

$$O_2^- + H_2O_2 \rightarrow OH^- + OH^- + O_2$$

Compared with hydrogen peroxide in its liquid form, VHP has been reported to be highly sporicidal at low concentrations. The action on VHP on spores appears to be the removal of protein from the spore coat. The use of *B. stearothermophilus* for the validation of VHP sterilization has been widely reported [11–14].

4. Radiation

Ionizing radiation can be delivered to a product to be sterilized either by gamma rays (cobalt-60 or cesium-137) or by accelerated electrons (electron beam). Although the method of depositing the radiation energy is different, the lethal effects are believed to be the same; gamma photons or E-beam electrons strike free electrons within the product which, in turn, strike other electrons resulting in ionization and the formation of free radicals.

Large organic molecules, such as DNA, appear to be particularly susceptible to ionizing radiation. Because DNA is the essential template of living systems, any major changes in the template result in an improperly built structure and, in the cell's case, nonviability. Direct effects of ionizing radiation on organic molecules appear to be bond scission and free radical formation. Indirect effects are attributed to reaction products produced by ionization and water (e.g., hydroxyl radicals and peroxides). These highly reactive compounds can interact with macromolecules and disrupt their activity. It appears, however, that the direct interactions with DNA are more important. The sensitivity of a microorganism to radiation is a function of the volume of DNA within the microorganism (more DNA means more sensitivity).

5. Filtration

Sterilization by filtration represents a special case relative to mechanism of action. In contrast with the other methods described thus far, filtration relies on the physical removal of microorganisms, rather than their destruction. Filtration, obviously limited to fluids, restrains microorganisms as the fluid passes through a filter. The mechanism of removal is a function of filter type. Depth filters, composed of fibers randomly pressed together, remove microorganisms by a combination of adsorption and entrapment in their internal structure. Membrane filters, formed by a controlled polymer precipitation process, can retain microorganisms by sieving, entrapment, or adsorption, or a combination thereof. Size exclusion, a combination of sieving and entrapment mechanisms, is one of the most reliable removal mechanisms operating in all fluids.

It is critical that the appropriate biological indicator and processing conditions are used to validate the sterilizing filter. For example, if adsorption was the predominant mechanism of removal in a membrane filter, changes in fluid physicochemical properties could serve to desorb the biological indicator cells and allow them to pass into the sterilized product.